Efficacy of Olyset® Duo, a pyriproxyfen and permethrin mixture bednet, against clinical malaria compared with standard nets in an area with highly pyrethroid-resistant vectors in rural Burkina Faso: a cluster-randomized controlled trial.

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Summary

Background Substantial reductions in malaria in sub-Saharan Africa have been achieved with massive deployment of long-lasting insecticidal nets (LLIN), but pyrethroid resistance threatens control. We assessed whether nets containing permethrin, a pyrethroid, and pyriproxyfen, an insect growth regulator (PPF-LLIN), provide incremental protection against malaria over LLINs and prompt treatment in an area of intense malaria transmission with pyrethroid-resistant vectors.

Methods In this two-arm, step-wedge, cluster-randomised, controlled, superiority trial, standard LLINs were replaced incrementally with PPF-LLINs in 40 rural clusters in Burkina Faso. In each cluster, 50 children, aged six months to five years, were followed by passive case detection for clinical malaria. Cross-sectional surveys were conducted at the start and end of the transmission seasons in 2014 and 2015. Monthly light trap collections were performed indoors to estimate vector densities. Primary endpoints were clinical malaria incidence measured by passive case detection and entomological inoculation rate (EIR). Analyses were adjusted for clustering and for month and health centre. The trial is registered as ISRCTN21853394.

Findings 1,980 children were enrolled in the cohort in 2014 and 2,157 in 2015. At the end of the study >99% of children slept under a bednet. Overall malaria incidence rate ratio was 0·88 (95% CI 0·77-0·99, p=0·04) for PPF-LLINs compared with standard LLINs. The *Plasmodium falciparum* parasite rate at the end of 2014 was similar in both study arms (odds ratio, OR=0·93 (0·74-1·15), p=0·50), although there were fewer cases of moderate anaemia in the PPF-LLIN arm (OR=0·48 (0·24-0·96), p=0·04). PPF-LLINs resulted in lower sporozoite rates in the vectors (OR=0·62 (0·47-0·83), p=0·001) and lower EIR compared with LLINs (relative risk=0·49 (0·32-0·66), p<0.001).

Interpretation PPF-LLINs provided greater protection against clinical malaria than standard LLINs. In areas with intense transmission and pyrethroid-resistant vectors PPF-LLINs could provide additional protection against clinical malaria.

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Introduction

There have been considerable gains in malaria control in sub-Saharan Africa with malaria prevalence dropping by half and the incidence of clinical disease falling by 40% from 2000-15.[1] This was achieved largely by the massive scaling-up of long-lasting insecticidal nets (LLIN) and indoor residual spraying (IRS). Yet according to the World Health Organization (WHO), despite the large-scale deployment of LLINs the decline in malaria incidence and malaria mortality has stalled, and these are even increasing in a few countries.[2] Burkina Faso, with more than 10 million uncomplicated cases of malaria annually, is one of 20 sub-Saharan countries where malaria cases increased between 2015 and 2016. It is also one of the few countries with no significant association between LLIN ownership and reduction in child mortality .[3] Even though approximately 90% of households in 2013 owned at least one LLIN, [4,5] national surveys show little impact on *Plasmodium falciparum* prevalence, with 61% of children aged six months to five years infected in 2014.[5] One possible reason for the lack of impact in the past and the rising number of cases in recent years is the increasing insecticide resistance in vectors seen in Burkina Faso[6] and across most of sub-Saharan Africa.[7]

Formulations are being developed for treating LLINs with a pyrethroid in combination with another active ingredient, such as pyriproxyfen to increase their effectiveness.[8-10] Pyriproxyfen is an insect growth regulator recommended for vector control by WHO because it is effective at extremely low concentrations and is safe for people,[11] and has a different mode of action to other classes of insecticide used for vector control. Pyriproxyfen is primarily used as a larvicide, preventing metamorphosis of pupae into adults, and can be effective for five to nine months after initial treatment.[12,13] In addition, it can sterilise adult mosquitoes, or at least reduce their fecundity and longevity.[14-16]

In experimental hut trials, polyethylene nets treated with a combination of permethrin and pyriproxyfen (PPF-LLIN; trade name Olyset® Duo, Sumitomo Chemical, Japan) were associated with higher mortality and reduced blood feeding in pyrethroid-resistant *Anopheles gambiae* s.s. compared to permethrin-only LLINs (Olyset®).[9,17] Further, PPF-LLINs sterilised blood-fed mosquitoes that survived exposure to pyrethroids.[17,8] Similar findings were observed in a pilot study where PPF-LLINs were introduced into village houses in Kenya.[10] There has, however, been no controlled trial demonstrating whether PPF-LLINs provide incremental protection against malaria beyond the current best practice of LLINs and prompt treatment. Building on current best practice, our study was designed to assess whether PPF-LLINs provide significant protection against clinical malaria to children less than five years old compared to permethrin-only ("standard") LLINs.

This is the first clinical trial to measure the protective efficacy of a bednet containing a mixture of two active ingredients with different modes of action. A net containing permethrin and piperonyl butoxide (PBO) has been tested recently in a clinical trial.[18] PBO is a synergist which increases the toxicity of permethrin but is not insecticidal. The use of mixtures of active ingredients is considered to be a better alternative to managing insecticide-resistant vectors since the chances of finding vectors resistant to both active ingredients is likely to be low, providing the enhanced detoxification of permethrin by resistant mosquitoes does not affect the other active ingredient.

Methods

Study design and participants

A detailed description of the study protocol has been reported previously.[19] This was a two-arm, step-wedge, cluster-randomised, controlled, superiority trial. The study took place in 2014-2015 in the south-east of Banfora town (10·6400° N, 4·7588° W, Figure 1), in Burkina

Faso, a country where malaria is highly endemic.[20] There is a high level of resistance to permethrin in the study site, with discriminating dose assays designed to kill 100% of susceptible mosquitoes killing only 13.4-19·2% *A. gambiae s.l.* in 2014 and 1-19·8% in 2015 (Supplementary material D).

The study design is shown in Figures 2 and 3. Prior to any study activity, village level permission was sought after sensitisation meetings attended by village community leaders and local health staff. A census of the study villages was carried out in 2013. Consenting villages were grouped into 40 village clusters consisting of one to four neighbouring villages.

A census was conducted in the study area to produce a list of all children fulfilling the age criteria. From this children were randomly selected for enrolment in the cross sectional survey. Four cross-sectional surveys were conducted: the first survey at the start of the transmission season in June-July 2014 defined the cohort of children enrolled in the study, with an average of 50 children per cluster and approximately equal numbers aged 6-35 and 36-60 months. A second survey was performed at the end of the first year of the study in December 2014, when equal numbers of clusters were in each of the study arms (see below). This survey included the cohort children and an additional minimum of 50 randomly-selected children per cluster (stratified by age). At the third survey at the beginning of the transmission season in May-July 2015, children who had dropped out of the study were replaced, as far as possible, by children of a similar age. A further additional minimum of 50 randomly-selected children per cluster were also included. A fourth and final survey was done at the end of the study in December 2015, in the cohort children and a further additional minimum of 50 randomly-selected children per cluster.

Written informed consent was obtained from each net recipient, before net donation and exchange. An additional signed informed consent was required from parents/legally acceptable representatives of children aged six months to five years who participated in the clinical assessments.

Randomisation and masking

The provision of bednets to study clusters is shown in Figure 3. At the start of the malaria transmission season in June 2014, five randomly-selected clusters of villages were provided with PPF-LLINs and the remaining 35 village clusters with LLINs (Figure 3 and supplementary material Figure A1). Subsequently, five clusters were randomly-selected to replace the LLINs with PPF-LLINs monthly from July to September 2014, so that by the end of 2014 an equal number of clusters were in each study arm. In 2015, PPF-LLINs were deployed in a similar fashion from May to September. This step-wedge design was adopted since it represents the type of deployment used by net distribution programmes. Random selection was performed using Stata (StataCorp. 2007. Stata Statistical Software: Release 10. College Station, TX: StataCorp LP).

Observer bias was reduced where feasible: both types of nets were of a similar shape, size and colour, and blood films were read by microscopists blinded to the identity and intervention status of the subjects.

Procedures

Interventions

White 'extra family' size rectangular nets (1·8 m wide × 1·9 m long × 1·5m high) containing 2% w/w permethrin incorporated into polyethylene fibres (Olyset® nets, Sumitomo Chemical, Japan) were distributed to achieve one LLIN per bed/sleeping place at the

beginning of the transmission season in 2014. PPF-LLINs were the same size as standard LLINs and contained 2% w/w permethrin and 1% w/w pyriproxyfen incorporated into polyethylene fibres (Olyset® Duo, Sumitomo Chemical, Japan). The chemical content of 30 randomly selected LLIN and 30 PPF-LLIN was checked using high-performance liquid chromatography at the Liverpool School of Tropical Medicine which confirmed the target doses (Supplementary material E1). PPF-LLINs were stored and distributed in a similar manner to the standard LLINs. PPF-LLINs were exchanged for standard LLINs at the household level. Government Roll Back Malaria information, education and communication procedures were followed to encourage correct net use and maintenance for both type of nets.

Clinical assessments

Passive case detection was used to measure clinical malaria incidence in the cohort children. Parents or carers of cohort children were encouraged to take their child to the nearest local health facility if the child was unwell. Clinical malaria was defined as a child presenting at a government health clinic with an axillary temperature of 37·5°C or more, or a history of fever in the past 48 h, together with the presence of *Plasmodium falciparum* parasites of any density detected by rapid diagnostic test (RDT, Paracheck Pf, Orchid Biomedical Systems, Goa, India) in the absence of other detectable cause of fever. To standardise data collection in the six health centres we posted one trained study nurse to each facility. At the health facilities these nurses carried out malaria diagnosis using RDTs, but also prepared blood slides that were later read at the CNRFP lab in Banfora. These nurses also communicated regularly with their managers so that stock outs were prevented. To facilitate documentation of all consultations by study children, all enrolled children were issued with enumerated photo identity cards. The child's study number, initials and village code were recorded for all clinical events on the case report form. Issues related to access and utilization rates by the public were mitigated by meeting the costs due to attendance for all the study participants.

In addition, we used anonymised medical records from three health facilities located outside the main study area valley to document clinical malaria based on positive RDTs in children aged six months to five years living in villages outside the study area. Although bednets were not provided by the study team to this area during the study, they were distributed during a nationwide bednet distribution campaign in 2014. Data from these records provided a way of tracking temporal changes in malaria incidence in the absence of the intervention, caused by factors such as weather patterns. Comparing this trend with that in the study area, provided additional evidence for the scale of community-wide intervention effects.

During the four cross-sectional surveys, all cohort and additional children, a minimum of 50 randomly-selected children per cluster, were visited at home and examined clinically for obvious symptoms and signs of illness, and temperature. Finger-prick samples were collected for haemoglobin measurement using a portable spectrophotometer (HemoCue v2.1, Ängelholm, Sweden), and for thick blood films. Those who had fever (axillary temperature ≥37·5°C) or a history of fever in the past 48 h were tested with a malaria RDT and, if positive, referred to the nearest health facility.

Thick blood films were stained with Giemsa and examined under 1000 times magnification. Parasite counts were recorded per high-power field and 100 fields counted before a slide was declared negative. Parasite density was estimated assuming that one parasite per high power field equals 500 parasites per μL. Two slides were prepared from each individual and the best one assessed separately by two experienced microscopists, with discrepancies resolved by a third.

Entomological assessments

Exposure to malaria vector mosquitoes was assessed using Centers for Disease Control and Prevention light traps every four weeks from May to December in 2014 and 2015 in six randomly selected households in each of 20 clusters in each study arm (Figure 2). Light traps were positioned next to a single sleeper protected with a standard LLIN or PPF-LLIN. Mosquitoes were identified by microscopy and the numbers of *A. gambiae s.l.* and other anophelines recorded. The presence of sporozoites were identified using an enzyme-linked immunosorbent assay[21] and a randomly selected subset of *A. gambiae s.l.* females were typed to species by PCR.[22]

Ethics Statement

The study was done in accordance with the principles established in the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice and the Declaration of Helsinki (2000), whichever afforded the greater protection to the participants. The trial was approved by the Ethics Committee for Health Research, Burkina Faso, on May 13, 2014 (reference 2014-3-24) and by the School of Biological and Biomedical Sciences Ethics Committee, Durham University, UK on January 17, 2014. A Data Safety Monitoring Board reviewed the trial procedures and results. The only incentives given to households that participated in the trial were provision of LLINs, treatment of study children during the study, and fares to reach referral clinics (refunded by study staff on the basis of known tariffs).

Outcomes

There were two primary endpoints: one clinical and one entomological. The clinical primary endpoint was the incidence of clinical episodes of malaria among cohort children presenting at health facilities. The entomological primary endpoint was entomological inoculation rate (EIR), or more strictly the household density of infective anophelines.[23] Secondary endpoints were presence of malaria parasites, prevalence of high parasitaemia (≥5000 parasites per μL), haemoglobin concentration, and prevalence of moderate (haemoglobin <80 g/L) and severe

anaemia (haemoglobin <50 g/L), measured at the end of the transmission season in 2014, when equal numbers of clusters were in each of the study arms. Children in the cohort were visited at home monthly by project staff for the duration of the PCD and if the child was absent more than 50% of a given calendar month then their data were censored for that month.

Safety considerations

Adverse events (AEs) and serious adverse events (SAEs) were recorded in the study cohort and population during the study. The trial followed standard definitions for AE and SAE agreed by consensus of the Collaborating Centres of the WHO International Drug Monitoring Centre (Uppsala, Sweden). The occurrence of AEs was sought by non-directive questioning of the study children at monthly visits during the trial. AEs were also recorded if volunteered by study children or their parent/carer or noted by nurses through physical examination, laboratory test, or other assessments at any contact with the participant. In addition, we recorded pregnancy outcomes for all resident pregnant women, and asthma signs/symptoms in pre-identified asthmatic residents.

Statistical analyses

For the power calculation, we assumed an incidence of clinical episodes of malaria of 1·5 episodes/child/year and a coefficient of variation of 0·5.[19] The study was designed to detect a protective efficacy of 25% with PPF-LLIN compared with standard LLINs with 90% power and at the 5% level of significance.

The statistical analysis followed the statistical analysis plan written before completion of the trial. Baseline characteristics of the study area population and cohort were summarised by month of rollout of the PPIF-LLINs, and a flowchart created to show the inclusion of children throughout the study. The primary clinical endpoint was a comparison of the incidence of clinical episodes of malaria in children in the PPF-LLIN versus standard LLIN arms, measured by PCD and accounting for time at risk. After a case of malaria was treated, further attacks of malaria were censored for the following four weeks, since attacks within this period

may have been false positives resulting from non-malaria fever and a positive RDT response due to persistence of parasite antigen, not an active infection.

The number of clinical episodes, the child-years at risk and the incidence rate (calculated as the ratio of these quantities) were calculated for each month of the trial in each arm, and the intervention effectiveness obtained by subtracting the relative rate from unity. Confidence intervals for these effectiveness estimates were obtained using the approximations given by Bennett *et al.*[24] We used Poisson regression models with log-transformed child time at risk as an offset, with random effects to account for village cluster, and adjusted for month and health facility as fixed effect. We incorporated an interaction between arm and month to provide month-specific estimates, and we considered adjustment for potential confounders (age, when joined the cohort, coverage, cluster size). These findings should be interpreted cautiously since interaction tests are typically underpowered.[25] We explored how changing the threshold of parasite density might affect the effect estimates of the intervention (supplementary material, section B).

Secondary endpoints were reported by arm with means and standard deviations or numbers and percentages for continuous and categorical outcomes, respectively. Correspondingly, linear or logistic regression models were used to compare between arms, with village cluster as a random effect, and health facility as a fixed effect. We repeated the analyses stratified by age <30 or ≥30 months (defined *a priori*) and stratified by whether cohort or additional children (defined post-hoc).

Numbers of indoor-mosquitoes, sporozoite rates defined as the percentage of mosquitoes with infective malaria parasites assessed by ELISA, parous rates defined as the percentage of parous mosquitoes identified by dissection, and species composition were summarised by

arm, and compared using logistic or negative binomial regression models with village cluster as a random effect, and month and health facility as fixed effect. EIR is defined as the number of infective bites received per person during the transmission season. We estimated EIR for each cluster as follows:

$$EIR = HDM \times SPR \times n$$

where *HDM* is the household density of mosquitoes, which is estimated as the mean number of *A. gambiae* s.l. per trap per night, *SPR* is the sporozoite rate and *n* is the number of days in the transmission season, May-November (n = 214). Further details are given in the supplementary material (section C). We excluded light trap collections for the calendar month that PPF-LLINs were introduced into a village to allow for a delay in the impact of PPF on the vector population. Analyses were performed using Stata (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Role of the funding source

The funder of the study and net manufacturers had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. Nets were donated by Sumitomo Chemical Company Ltd.

Results

In total, 92 villages were approached to take part into the study, and, following community-level meetings to discuss the nature of the study and what would be required during the interventions and investigations, 91 villages agreed to participate.

In June 2014, 29,084 LLINs were distributed to cover the 30,608 sleeping places identified during the pre-study population census (24,357 LLIN and 4,727 PPF-LLIN), yielding an

overall coverage rate of 95%. Detailed coverage at cluster level is presented in supplemental material (supplementary material Table A1). Overall, coverage exceeded 80% across all the clusters except one where residents multiple absences from home for traditional gold mining activities made it impossible to deliver nets despite multiple attempts.

Figure 4 illustrates the flow of the cohort children through the trial, with some being lost (and some returning) and some exiting on reaching six years of age. At the first survey, 1,980 children were enrolled in the cohort, with 675 children added at the third survey to replace those lost or exited. By the fourth survey at the end of study, there were 2,148 children in the cohort. An average of 46-56 additional children per cluster were included in surveys two to four. At the first survey, 49% of cohort children were female and the mean age was 35 months (Table 1). Baseline characteristics were roughly equivalent across clusters (grouped by month of roll out of PPF-LLINs), although there was a large variation in RDT positivity between clusters. Replacement cohort children at the third survey had broadly similar characteristics to those already enrolled (supplementary material Table A2).

Overall incidence of clinical malaria was 1·5 versus 2·0 per child year at risk in the PPF-LLIN versus LLIN arms, respectively (Figure 5 and Table 2), corresponding to an unadjusted relative risk of 0·72 (95% CI 0·66-0·78) (supplementary material Table A3). This is a biased estimate of effect because in both years incidence was higher in the first part of the transmission season (when there were more children in the LLIN arm) than in the September-December period (when there were more in the PPF-LLIN arm). Various analyses adjusting for month gave lower estimates of the reduction in incidence (Table A3), with the primary analysis specified in the analytical plan, that included adjustment for month and health facility, providing weak but significant evidence for a difference between the arms (rate ratio, RR=0·88 (0·77-0·99); p=0·04; Table 2). Similar results were obtained adjusting for potential

confounders (Table A3). The number of malaria cases reported outside the study area was similar in the first and second years, indicating no change in the intensity of malaria transmission between the two years (supplementary material Figure A2). The specificity of clinical malaria definitions increases with the use of a higher parasite density threshold, as expected. Nevertheless with this dataset, the use of a more specific case definition does not lead to a higher effectiveness estimate (supplementary material, section B).

At the second survey, when there was an equal number of clusters in each study arm, there was no evidence of a difference by arm in the presence of malaria parasites (61% versus 62% for PPF-LLINs versus standard LLINs, respectively; odds ratio (OR)=0·93, 95% CI 0·74-1·15, p=0·50; Table 3). There were trends towards lower prevalence of high parasitaemia and higher haemoglobin concentration in the PPF-LLIN versus standard LLIN arm although the p-values were large (18% versus 20%, respectively; OR=0·87, 0·73-1·02, p=0·09; and mean 103·5 g/L versus 101·4 g/L, respectively; difference=1·8, -0·4-3·9 g/L, p=0·11). Significantly higher levels of haemoglobin were observed in children under 30 months of age using PPF-LLINs (99·3 g/L) compared with those using standard LLINs (95·4 g/L) (difference=3·5, 0·9-6·1 g/L, p=0·008, supplementary material Table A4). Overall there was a lower prevalence of moderate anaemia in the PPF-LLIN versus standard LLIN arm (3% versus 6%, respectively; OR=0·48, 0·24-0·96, p=0·04). There was also an indication that among the additional (non-cohort) children, there were fewer children with high parasite densities in the arm with PPF-LLINs than those with LLINs (20% versus 24% respectively, OR=0·80, 0·61-0·96, p=0·02, supplementary material Table A5).

99% of the planned entomological collections (3090/3123) were successfully carried out (in the other cases the houses were locked). Female *Anopheles gambiae* s.l. were present in 55%

(1687/3090) of light trap collections (Table 4). Of those mosquitoes collected, 91% (41,548/45,414) were anophelines, of which 98 % (40,587/41,548) were *A. gambiae* s.l.; all the rest were culicines. Of the *A. gambiae* s.l. identified to species, 87% (11,852/13,584) were *A. gambiae s.s.*, 11% (1,519/13,584) *A. coluzzii*, <1 % hybrids (9/13,584) and 2% (204/13,584) *A. arabiensis*. The entomological indices were strongly seasonal (Figure 6), with a strong wet-season peak in vector densities each year and an increase in parous rates at the end of the wet season. The seasonal pattern in the sporozoite rate differed between years, with patterns of estimated EIRs dominated by the trends in vector densities. As is typical of settings with seasonal malaria, the vector populations were dominated by young, recently emerged mosquitoes early in the transmission season, while older, parous, mosquitoes (that are more likely to harbour sporozoites) formed a higher proportion of the population later in the season (Figure 6). Consequently, the parous and sporozoite rates were lower in the initial period of the trial (when all clusters were in the LLIN arm) than in the last few months of the trial (when all were in the PPF-LLIN arm). Because of this, average values of the entomological indices, computed without allowing for the differential representation over time of the two arms, were similar in the two arms, even though the contemporaneous comparisons demonstrated substantially lower densities, parous rates, and sporozoite rates in the PPF-LLIN arm at almost all time points (Figure 6). Adjustment for the differential contributions at different time-periods was important in providing coherent estimates of the overall entomological impacts (Table 4).

There was no difference in the composition of *A. gambiae s.s.* and *A. coluzzii*, the two commonest vectors, between intervention arms (OR=1·19, 0·94-1·51), p=0·14). Densities of female *A gambiae* s.l., however, were lower in the PPF-LLIN versus standard LLIN arm (mean 14 versus 12/trap/night, respectively; ratio ratio=0·78, 0·68-0·89; p<0·001), and the

parous rates were also lower (OR=0·69, 0·52-0·91; p=0·009). Similarly we found lower sporozoite rates in the PPF-LLIN versus standard LLIN arm (3% versus 4%, respectively; OR=0·62, 0·47-0·83; p=0·001). The EIR was 58% lower in the PPF-LLIN versus standard LLIN arm (EIR=42, 32-52) versus 85 (63-108) infective bites/season, respectively (RR=0·49, 0·32-0·66).

Adverse events are described in the supplementary material (Tables A5-A8). There were no indications of any adverse events associated with the PPF-LLINs.

Discussion

We present evidence that in a rural area of Burkina Faso with high bednet coverage, high levels of malaria transmission and the presence of vectors highly resistant to permethrin, children sleeping under PPF-LLINs had 12% fewer clinical episodes of malaria than those with standard LLINs. Moreover, children with PPF-LLINs were 52% less likely to be moderately anaemic than those with conventional nets. Such a reduction in anaemia is clinically relevant, especially since malaria anaemia is a major cause of mortality in children under two years old.[26] The entomological data suggest that protection arose from the mass killing of malaria vectors reducing the densities and reduced survival of the vector population supporting the evidence for the protective effect of PPF-LLINs. There was a highly significant 22% reduction in vector numbers and 31% lower odds of finding parous (older) mosquitoes in clusters with PPF-LLIN, consistent with the anticipated mass effect of the PPF-LLINs. This corresponded to a 58% reduction in EIR in the PPF-LLIN arm compared to standard LLINs.

At the same time, there was no measurable effect of PPF-LLINs on malaria parasite prevalence, consistent with other studies that show that prevalence is only weakly related to EIR in the observed range of approximately 40-80 infective bites per year in unprotected

individuals.[27] The high parasite prevalence of around 60% diagnosed by microscopy, despite levels of bednet ownership much higher than reported for most countries[2], probably means that nearly every child is infected with parasites.[28] Most of them were presumably harbouring infections that had persisted since before the trial, and which could therefore not have been averted by the new nets. This high prevalence also led to a downward bias in the estimate of effectiveness against clinical malaria because the numbers of clinical malaria cases recorded in both arms of the trial are inflated by cases of fever of non-malarial aetiology, diagnosed as malaria because of incidental parasitaemia, suggesting that the true efficacy against disease is likely to be higher than 12% (Supplementary Information B).

The mode of action of the PPF-LLIN is complex since it relies on a combination of factors: (1) an intact bednet correctly used provides a physical barrier against blood-seeking mosquitoes, (2) the permethrin on the net has excito-repellent properties with rapid knock down and kill of pyrethroid susceptible mosquitoes, (3) pyriproxyfen reduces both adult longevity and reproductive outputs of female mosquitoes. Although the permethrin concentration on the two net types is the same, the bleed rates are greater in the PPF-LLIN than the LLIN. This means that the likely surface concentration of permethrin is higher on the PPF-LLIN than the LLIN. This is supported by studies that have found higher levels of mortality in pyrethroid-resistant mosquitoes exposed to PPF-LLINs than conventional LLINs.[9,17,29] Another cluster randomised trial is currently on-going in the same district to compare the durability of PPF-LLINs and LLINs.[30] This may enable us disentangle which factor(s) account for the better protection provided by the PPF-LLIN. At present, we cannot be certain whether the protective effect is due to increased concentrations of permethrin on the fibre or the PPF or both.

There are few opportunities to manage insecticide resistance in *Anopheles* mosquitoes in sub-Saharan Africa where LLINs are often the sole malaria preventative tool and where only pyrethroids are being used to treat these nets. The use of mixtures on nets, like the PPF-LLIN net, is an advance on pyrethroid-only nets, providing these nets are cost effective and providing that there is no cross resistance between the two chemical classes. There is, however, evidence to suggest that the elevated levels of cytochrome P450s found in pyrethroid resistant *Anopheles* populations may reduce the bioefficacy of pyriproxyfen.[29] Thus the exceptionally high levels of permethrin resistance in the study site will reduce the impact of both the pyrethroid and potentially the PPF component of the PPF-LLINs and a greater clinical impact of PPF-LLINs is likely in areas with lower levels of pyrethroid resistance and lower levels of vectorial capacity. In the future, in areas of high pyrethroid resistance, the use of nets treated with mixtures of active ingredients with different modes of action and which do not include pyrethroids, should be explored.

There are at least two limitations to this study. Firstly, although the communities were masked to the interventions, it is possible that study participants would view a new net as better than an old one, potentially resulting in under-reporting of clinical cases in the PPF-group. Secondly, the large-scale use of PPF LLIN may have reduced the number of malaria vectors dispersing from these clusters into adjacent clusters with LLIN, and this will be explored in a later analysis.

The rapid spread of pyrethroid resistance in African vectors is a cause of concern. Although we are currently lacking direct evidence that this is interfering with malaria control, it is likely that this will become an issue in future. At present the only strong indication that resistance is interfering with malaria control comes from a two-armed trial of pyrethroid nets; where greater clinical protection was achieved when PBO was added to the nets compared with

standard pyrethroid only nets.[18] PBO is a synergist that potentiates the insecticidal activity of the permethrin, but cannot fully restore susceptibility against all resistant populations. There is therefore an urgent need to find alternative strategies for dealing with insecticide resistance. We selected the PPF-LLIN as the most commercially advanced dual action combination net, and have conducted the trial in one of the most challenging settings in sub-Saharan Africa; where pyrethroid resistance in vectors is high and extremely common and where vectorial capacity is one of the highest in the region. The trial showed that substantial numbers of malaria cases could be averted by these nets in such areas. In Burkina Faso 10,061,318 uncomplicated malaria cases were recorded in 2017[31], thus a 12% reduction in malaria incidence seen with PPF-LLINs would equate to 1.2 million cases averted. PPF-LLINs are a promising intervention against malaria in sub-Saharan Africa. It should, however, be appreciated that deployment of mixture nets should not result in lower coverage.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SWL, SN, MP, TS, HR and ABT conceived and designed the study, and drafted the manuscript. BF, MP, TS and SWL designed the analytical plan. The random allocation sequence and assignment of participants to the interventions were generated by MP. AlO, DO and SC led the field implementation of the study. ECB supervised the nets storage and distribution. SS implemented the quality assurance plan for the trial. AD and INO were responsible for the clinical laboratory assessments. The entomology was coordinated by SN and MWG. MWG led the entomological analysis and NG conducted the insecticide bioassays. AmO led the data management team. BF, FV and TS performed the statistical analyses. All authors read and approved the final manuscript.

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Table 1. Characteristics of children enrolled into the cohort at the first survey (June/July 2014).

	Month of rollout of PPF-LLINs								
Characteristics	**June 2014**	**July 2014**	**Aug 2014**	**Sept 2014**	**June 2015**	**July 2015**	**Aug 2015**	**Sept 2015**	**Total**
Children enrolled, N	274	215	270	256	216	220	251	278	1980
Female, N (%)	118/274 (43%)	105/215 (49%)	126/270 (47%)	131/256 (51%)	108/216 (50%)	99/220 (45%)	130/251 (52%)	145/278 (52%)	962/1980 (49%)
Age (months), median (IQR)	34 (22,48)	37 (21,48)	35 (22,50)	34 (21,49)	35 (23,49)	35 (23,49)	35 (22,47)	35 (22,48)	35 (22,48)
Sleeps under a mosquito net, N (%)	254/274 (93%)	184/215 (86%)	261/270 (97%)	238/256 (93%)	209/216 (97%)	197/220 (90%)	217/251 (86%)	268/278 (96%)	1828/1980 (92%)
Took anti-malarials in last 14 days, N (%)	21/274 (8%)	13/215 (6%)	12/270 (4%)	26/256 (10%)	11/216 (5%)	15/220 (7%)	28/251 (11%)	17/278 (6%)	143/1980 (7%)
Sick with a fever during previous 48 hours, N (%)	42/274 (15%)	24/215 (11%)	35/270 (13%)	29/256 (11%)	19/216 (9%)	33/220 (15%)	33/251 (13%)	35/278 (13%)	250/1980 (13%)
Axillary temperatures (°C), median (IQR)	36·7 (36.4,37.1)	36·6 (36.4,36.9)	36·7 (36.4,36.9)	36·7 (36.5,36.9)	36·6 (36.2,36.9)	36·6 (36.2-36.9)	36·4 (36.2,36.9)	36·5 (36.1,36.9)	36·6 (36.3,36.0
Positive rapid diagnostic test, N (%)	38/52 (73%)	16/28 (57%)	32/50 (64%)	17/38 (45%)	16/20 (80%)	38/49 (78%)	26/36 (72%)	43/48 (90%)	226/321 (70%)
Presence of *Plasmodium falciparum* parasites by microscopy, N (%)	120/273 (44%)	93/182 (51%)	141/267 (53%)	138/249 (55%)	96/207 (46%)	114/220 (52%)	141/248 (57%)	138/272 (51%)	981/1918 (51%)
>5000 *Plasmodium falciparum* parasites per µL, N (%)	36/273 (13%)	26/182 (14%)	31/267 (12%)	36/249 (14%)	35/207 (17%)	34/220 (15%)	43/248 (17%)	30/272 (11%)	271/1918 (14%)
Plasmodium falciparum parasite density (per µl), geometric mean (geometric SD) of non-zero values	1475 (7·2)	1988 (5·0)	1378 (5·5)	1697 (6·3)	2479 (6·6)	1823 (6·9)	1784 (6·1)	1474 (5·3)	1698 (6·1)
Presence of *Plasmodium falciparum* gametocytes, N (%)	49/273 (18%)	33/182 (18%)	64/267 (24%)	51/249 (20%)	42/207 (20%)	38/220 (17%)	54/248 (22%)	49/272 (18%)	380/1918 (20%)
Haemoglobin level (g/L), median (IQR)	103·0 (91.0,113.0)	101.0 (91.0,110.0)	103.0 (94.0,111.0)	107.0 (101.0-113.0)	103.0 (94.5-115.0)	98.0 (91.0,109.0)	101.0 (96.0,107.0)	102.0 (94.0,111.0)	102.0 (94.0,111.0)

Moderate anaemia (haemoglobin <80 g/L), N (%)	27/266 (10%)	20/209 (10%)	22/270 (8%)	8/241 (3%)	11/156 (7%)	19/220 (9%)	8/173 (5%)	16/265 (6%)	131/1800 (7%)
Severe anaemia (haemoglobin <50 g/L), N (%)	0/266 (0%)	0/209 (0%)	0/270 (0%)	0/241 (0%)	1/156 (1%)	0/220 (0%)	1/173 (1%)	0/265 (0%)	2/1800 (0%)

IQR, interquartile range.

Table 2. Incidence of clinical malaria in the cohort.

Study year	Month	Number of malaria episodes reported		Number of years of exposure		Number of events per child exposure year		% reduction [1]	Rate ratio (95% CI) [1,2]	Model-based rate ratio (95% CI) [1,3]
		Standard LLINs	*PPF-LLINs*	*Standard LLINs*	*PPF-LLINs*	*Standard LLINs*	*PPF-LLINs*			
2014	Jun	33		79		0·4				
	Jul	454		123		3·7				
	Aug	244	43	103	23	2·4	1·9	22	0·78 (0·54,1·13)	0·83 (0·59,1·17)
	Sep	177	66	79	39	2·3	1·7	25	0·75 (0·55,1·01)	0·82 (0·60,1·10)
	Oct	212	155	81	63	2·6	2·5	7	0·93 (0·75,1·16)	0·93 (0·74,1·16)
	Nov	193	170	78	81	2·5	2·1	16	0·84 (0·68,1·05)	0·90 (0·72,1·12)
	Dec	111	92	80	84	1·4	1·1	20	0·80 (0·59,1·07)	0·85 (0·63,1·13)
2015	May	15	14	789	82	0·2	0·2	10	0·90 (0·42,1·94)	0·95 (0·46,1·99)
	Jun	42	50	59	77	0·7	0·6	9	0·91 (0·59,1·41)	0·91 (0·60,1·38)
	Jul	146	223	54	99	2·7	2·3	17	0·83 (0·66,1·04)	0·79 (0·63,1·00)
	Aug	64	266	29	123	2·2	2·2	1	0·99 (0·73,1·34)	0·98 (0·73,1·32)
	Sep		271		139		1·9			
	Oct		337		166		2·0			
	Nov		304		185		1·6			
	Dec		56		189		0·3			
Overall		**1691**	**2047**	**844**	**1351**	**2·0**	**1·5**	**24**	**0·76 (0·71,0·81)**	**0·88 (0·77,0·99) p=0·04**

Clinical malaria is the primary endpoint of the trial, defined as axillary temperature of ≥37.5 °C or history of fever in the past 48 hours, plus positive RDT, detected through PCD, excluding children the month of and after the introduction of the intervention. [1] For PPF-LLINs versus standard LLINs. [2] Confidence intervals estimated using methods of Bennett *et al.* [24] [3] Poisson model with offset for exposure time (natural log transformed), with random intercept for cluster, and month and health facility as fixed effect (with an interaction between month and randomisation arm to obtain the month-specific results). Test for interaction between arm and month, p=0.97. Test for month, p<0.001; test for health facility, p<0.001 (in model without interaction between arm and month). P-value is from aWald test.

Table 3. Characteristics of children at the cross-sectional surveys, by arm.

Characteristics	Survey 1 (June/July 2014)		Survey 2 (December 2014)			Survey 3 (May-July 2015)		Survey 4 (December 2015)	
	LLINs (n=1681)	PPF-LLINs (n=0)	LLINs (n=1799)	PPF-LLINs (n=1874)	Odds ratio (OR) or coefficient (95% CI; p) [1]	LLINs (n=1469)	PPF-LLINs (n=1984)	LLINs (n=0)	PPF-LLINs (n=4100)
Female, N (%)	829/1681 (49%)	-	903/1799 (50%)	923/1874 (49%)	-	713/1469 (49%)	958/1984 (48%)	-	2004/4100 (49%)
Age (months), mean (SD)	35 (15·0)	-	41 (15·0)	41 (15·2)	-	38 (13·6)	37 (14·0)	-	43 (14·4)
Slept under a mosquito net, N (%)	1549/1681 (92%)	-	1793/1799 (>99%)	1867/1874 (>99%)	-	1411/1423 (99%)	1901/1910 (>99%)	-	3909/3913 (>99%)
Presence of *Plasmodium falciparum* parasites by microscopy, N (%)	851/1627 (52%)	-	1096/1761 (62%)	1124/1843 (61%)	OR=0·93 (0·74,1·15; p=0·50)	604/1388 (44%)	757/1854 (41%)	-	2159/3758 (57%)
>5000 *Plasmodium falciparum* parasites per µl, N (%)	229/1627 (14%)	-	358/1761 (20%)	338/1843 (18%)	OR=0·87 (0·73,1·02; p=0·09)	172/1388 (12%)	208/1854 (11%)	-	627/3758 (17%)
Haemoglobin level (g/L), mean (SD)	101·5 (13·47)	-	101·4 (13·86)	103·5 (11·74)	Coefficient= 1·8 (-0·4,3·9; p=0·11)	104·3 (12·44)	105·5 (11·58)	-	103·7 (10·65)
Moderate anaemia (haemoglobin <80 g/L), N (%)	104/1511 (7%)	-	113/1768 (6%)	54/1782 (3%)	OR=0·48 (0·24,0·96; p=0·04)	62/1420 (4%)	51/1834 (3%)	-	62/3726 (2%)
Severe anaemia (haemoglobin <50 g/L), N (%)	2/1511 (0%)	-	7/1768 (0%)	0/1782 (0%)	Not estimable	1/1420 (0%)	0/1834 (0%)	-	0/3726 (0%)

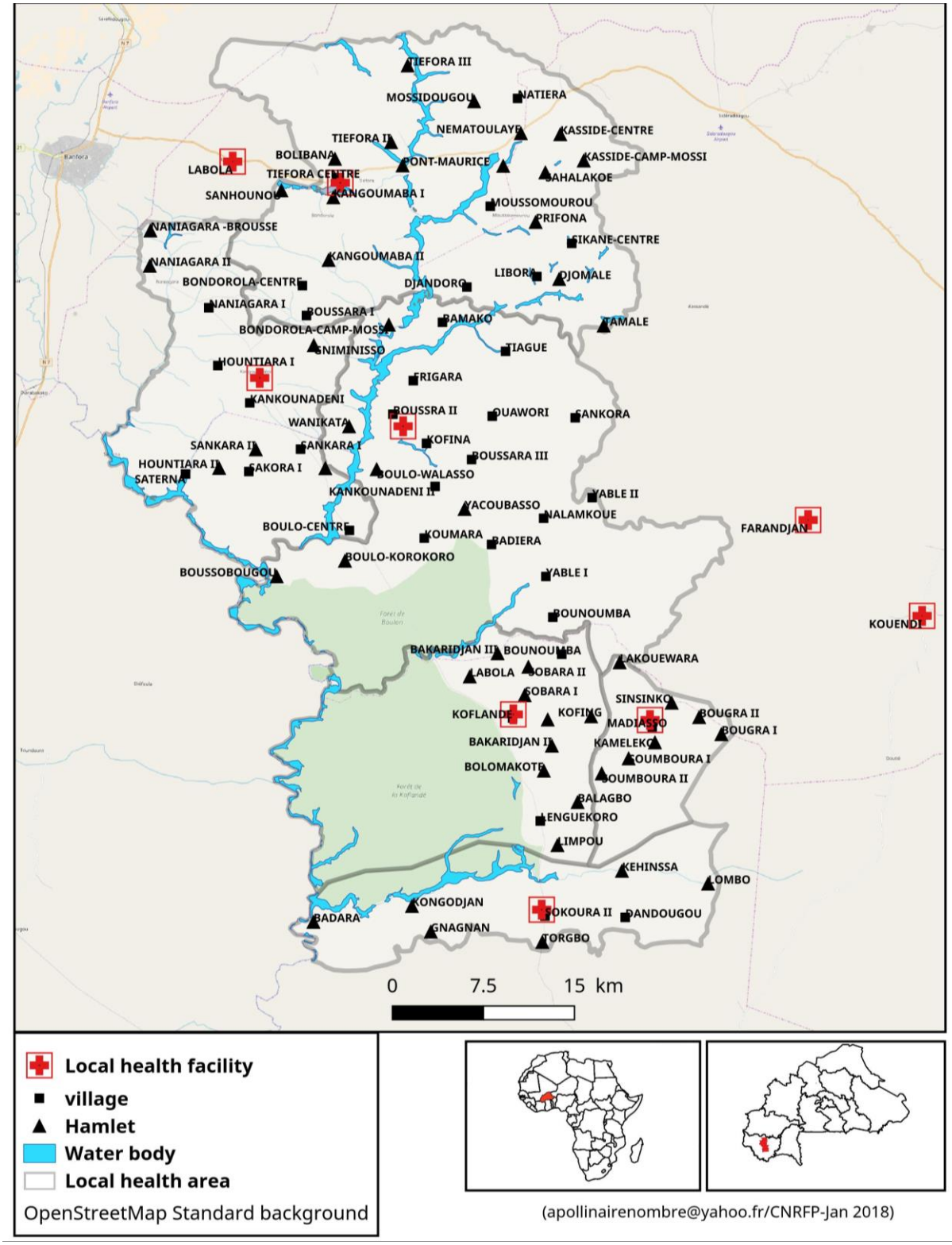
Includes cohort and additional children, but excluding children during the month of and month after the introduction of the intervention, therefore for example the numbers of children shown in the first survey differ from those shown in Table 1 (see Figure 4). Rows shaded in blue are secondary endpoints of the trial. [1] Odds ratio or coefficient with 95% confidence interval and p-value for PPF-LLINs versus standard LLINs, using logistic and linear regression models for categorical and continuous variables, respectively, with cluster as a random effect and health facility as a fixed effect (only done for secondary endpoints at survey 2 when there were equal numbers of clusters in each of the study arms).

Table 4. Entomological results.

Parameter	Unadjusted estimates		Adjusted estimates [1]			
	Standard LLINs	PPF-LLINs	Standard LLINs	PPF-LLINs	Odds ratio (OR) or rate ratio (RR) (95% CI)	P
Number of light trap collections	1248	1842				
Number of female *A. gambiae* s.l. collected (%)	144894/16785 (86%)	25820/28629 (90%)				
Species composition (of *A. gambiae s.l.*) [2]						
A. arabiensis	56/4774 (1%)	148/8810 (2%)				
A. coluzzii	403/4774 (8%)	1116/8810 (12%)				
A. coluzzii/A gambiae s.s.	5/4774 (<1%)	4/8810 (<1%)				
A gambiae s.s.	4310/4774 (90%)	7542/8810 (86%)				
A. coluzzii	403/4713 (9%)	1116/8658 (13%)			OR=1·19 (0·94-1·51)	p=0.14
Mean (SD or 95% CI) female *A. gambiae* s.l. collected per trap	12 (32)	14 (36)	9·4 (7·7-11·0)	7·3 (6·1-8·5)	RR=0·78 (0·68-0·89)	p<0·001
Parous rate (% or 95% CI)	625/1038 (60%)	1364/2198 (62%)	69% (64-74%)	61% (57-65%)	OR=0·69 (0·52-0·91)	p=0·009
Sporozoite rate (% or 95% CI) [3]	206/4858 (4%)	273/8935 (3%)	4·3% (3·4-5·1%)	2·7% (2·3-3·1%)	OR=0·62 (0·47-0·83)	p=0·001
Estimated entomological inoculation rate (EIR, 95% CI) [4]			85 (63-108)	42 (32-52)	RR=0·49 (0·32-0·66)	p<0.001

CI=confidence interval. SD=standard deviation. [1] Odds or rate ratio with 95% confidence interval and p-value for PPF-LLINs versus standard LLINs, using logistic regression models or negative binomial regression models, respectively, with cluster as a random effect, and month and health facility as fixed effect. For the parous rate, the mean number collected per trap and the sporozoite rate (the latter two required for the EIR estimation), corresponding proportions and means were estimated from the models, marginal across month and health facility, with a random effect of zero for the dispersion parameter. [2] Missing for 85 and 126 mosquitoes in the standard LLIN and PPF-LLIN arms, respectively. [3] Missing for one mosquito in the PPF-LLIN arm. [4] See methods for estimation details.

Figure 1. Map of the study area: Banfora, rural south-west Burkina Faso.



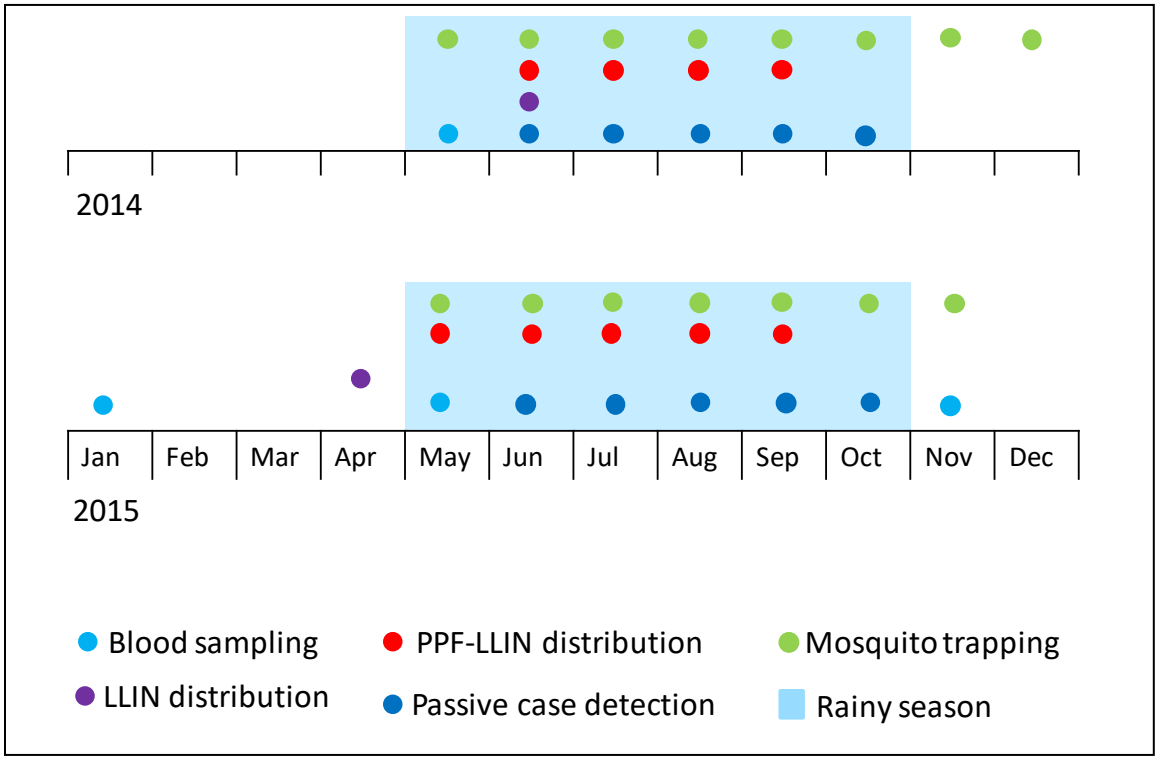


Figure 2. AvecNet study design.

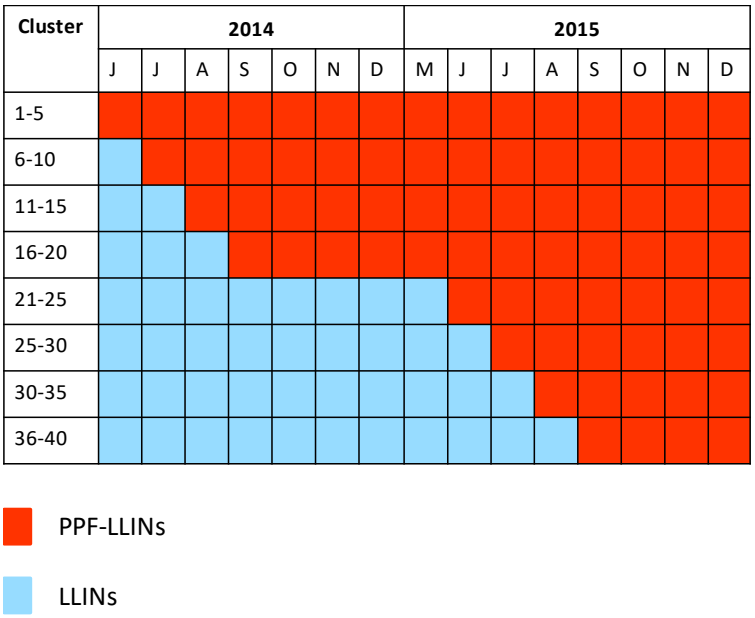
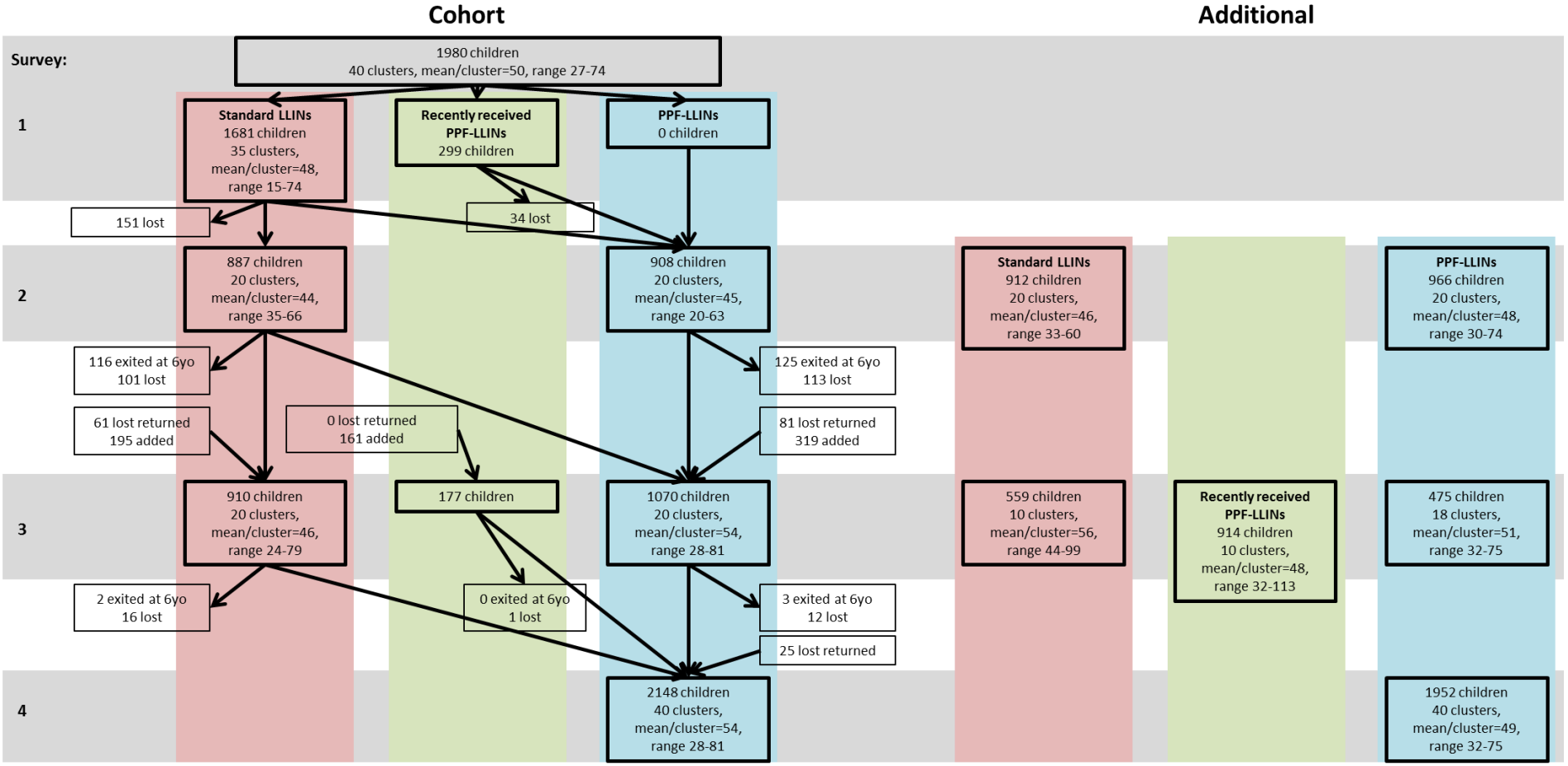


Figure 3. Step-wedge roll out of PPF-LLIN across the clusters.

Figure 4. Flow chart of cohort and additional children.



Grey horizontal bands indicate the four surveys that were conducted during the trial (see Figure 2). The figure is divided into the children who were enrolled into the cohort (left) and the children who were additionally surveyed (right). The red vertical bands illustrate children who were still in the standard long-lasting insecticidal nets (LLIN) arm, the green vertical bands illustrate children who had recently received the pyriproxyfen and permethrin combination long-lasting insecticidal nets (PPF-LLINs; and so would not be counted in the cross-sectional survey analyses; see Methods), and the blue vertical bands illustrate children who had received the PPF-LLINs (at least 2 months ago and therefore are included in the cross-sectional survey analyses). During the trial, some children exited due to turning 6 years old and some were lost; at the third survey, 675 children were added to the cohort to replace those exited or lost (see Methods).

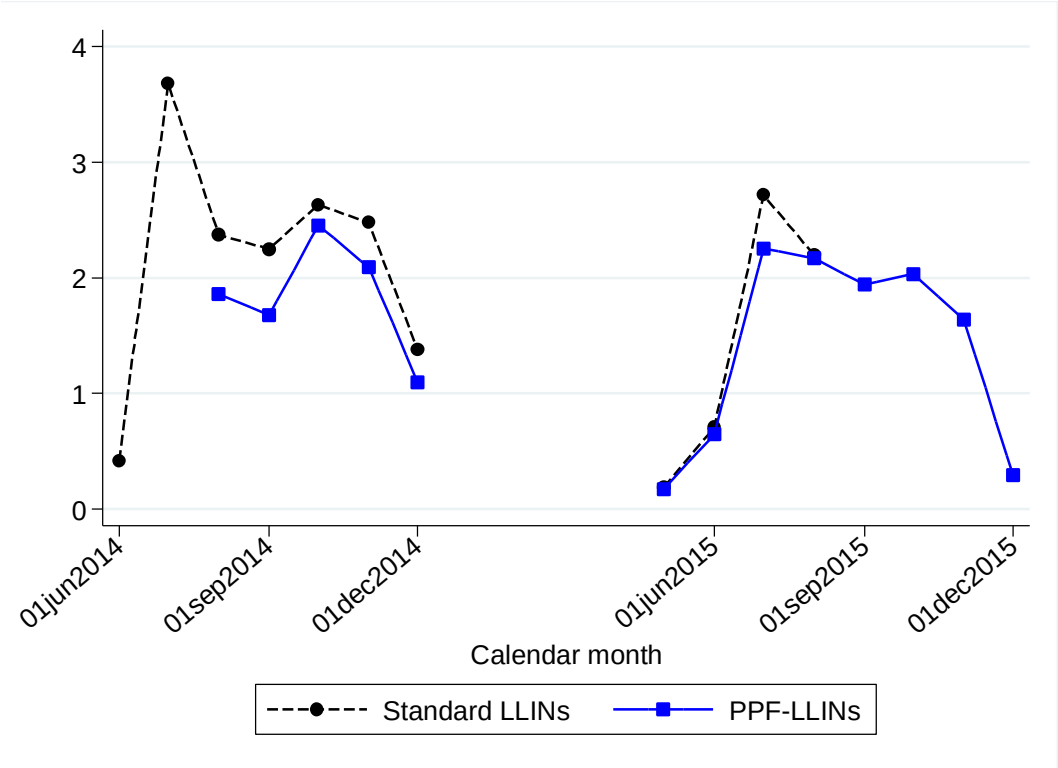


Figure 5. Incidence of clinical malaria in the cohort. Clinical malaria is the primary endpoint of the trial, defined as axillary temperature of ≥37.5 °C or history of fever in the past 48 hours, plus positive RDT, detected through PCD, excluding children the month of and after the introduction of the intervention.

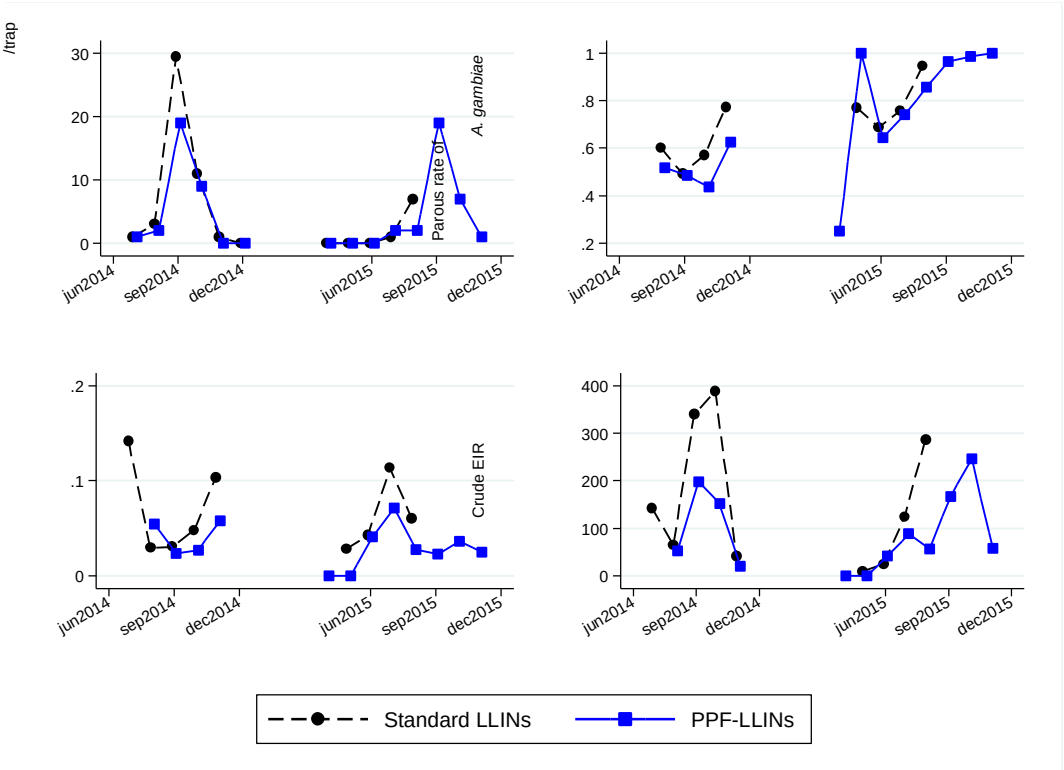


Figure 6. Entomology results. Excluding collections the month of the introduction of the intervention.